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ARMY MEDICAL RESEARCH LABORATORY

FORT KNOX, KENTUCKY

REPORT NO. 156
8 December 1954

EFFECT OF SOFT X-RAYS ON THE LUMINESCENCE EFFICIENCY OF BIOLOGICAL COMPOUNDS*

*Subtask under Biological and Medical Aspects of Ionizing Radiation,
AMRL Project No. 6-59-08-014, Subtask, Effects of Ionizing Radi-
ation.



RESEARCH AND DEVELOPMENT DIVISION
OFFICE OF THE SURGEON GENERAL
DEPARTMENT OF THE ARMY

Report No. 156

From Project No. 6-59-08-014

EFFECT OF SOFT X-RAYS ON THE
LUMINESCENCE EFFICIENCY OF BIOLOGICAL COMPOUNDS

by

W. H. Parr, J. G. Kereiakes and A. T. Krebs

from

Radiobiology Department

Submitted

23 August 1954

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Abstract;

This report involves the study of the luminescence efficiency of different biological compounds under high-energy gamma bombardment before and after 50 kv x-irradiation. After x-irradiation some compounds (crude catalase) showed no change; in other compounds (ascorbic acid) luminescence increased; and in others (glutathione) luminescence decreased. Parallel measurements made on the luminescence changes and chemical changes of the solute indicate that the luminescence curve does not follow closely the decomposition curve. These studies illustrate the influence of the radiation-induced decomposition products and are of importance in considering biological reactions induced by ionizing radiation.

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EFFECT OF SOFT X-RAYS ON THE
LUMINESCENCE EFFICIENCY OF BIOLOGICAL COMPOUNDS*

by

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*Subtask under Biological and Medical Aspects of Ionizing
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of Ionizing Radiation.

Report No. 156
Project No. 6-59-08-014
Subtask AMRL S-1
MEDEA

ABSTRACT

EFFECT OF SOFT X-RAYS ON THE LUMINESCENCE EFFICIENCY OF BIOLOGICAL COMPOUNDS

OBJECT

To study the luminescence efficiency of different biological compounds under high-energy gamma bombardment before and after 50 kv x-irradiation. Of prime importance is the possible role of the radiation-induced decomposition products as reflected by changes in the luminescence properties of the compounds.

RESULTS

In all cases the compounds quenched the solvent luminescence. After x-irradiation some compounds (crude catalase) showed no change in luminescence efficiency; in other compounds (ascorbic acid) luminescence increased; and in others (glutathione) luminescence decreased. The irradiation-effect dependence of luminescence on the concentration showed no change in certain compounds (crude catalase); a change increasing with concentration and approaching a saturation value in other compounds (glutathione); and a change going through a maximum with increasing concentration in others (ascorbic acid). Parallel measurements made on the luminescence changes and the chemical changes of the compound for various x-ray doses indicated that the luminescence does not follow closely the decomposition curve. After an extended period of irradiation, both the luminescence efficiency and the chemical concentration showed a 100% change.

The quenching of the solvent luminescence by the addition of the biological solute molecules is evidence of energy transport from solvent to solute molecules. The absence of any significant changes in the luminescence efficiency of x-irradiated catalase as compared to the non-irradiated catalase may indicate the absence of decomposition products. In the case of glutathione where the luminescence efficiency decreased, it may be considered that the decomposition products were able to increase the quenching of the

solution. With ascorbic acid, which showed an increase in luminescence efficiency (yet never surpassing that of the solvent), the decomposition products may be considered to have had less efficient quenching properties at certain concentrations. The difference observed between the luminescence changes and the concentration change of the compound for various x-ray doses points out the influence of the decomposition products on the luminescence characteristics of the compounds. The findings presented here are of importance in considering the biological reaction induced by ionizing radiation.

RECOMMENDATIONS

Other types of radiation should be studied to permit comparison with the 50 kv x-irradiation data presented here. Spectral curves should be obtained for the biological compound solutions under the various x-irradiation conditions in order to correlate the spectral changes with the observed luminescence intensity changes.

Submitted 23 August 1954 by:

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EFFECT OF SOFT X-RAYS ON THE LUMINESCENCE EFFICIENCY OF BIOLOGICAL COMPOUNDS

I. INTRODUCTION

Recent discussions on the role of excitations on the biological effects of radiation indicate a need for additional knowledge of energy transport and energy migration mechanisms in irradiated biological systems. New opportunities for an understanding of these mechanisms are offered in the studies of organic and inorganic liquid scintillators by Kallman and Furst (1), Reynolds (2), Birks (3), Bowen (4), Harrison (5), Swank (6), and others. Birks, and Kallman and Furst have advanced possible mechanism theories in recent publications

With the above considerations as background, studies of the effect of 50 kv x-rays on the luminescence efficiency of biological compound solutions were started. Previous work on irradiated biological systems has been generally concerned with chemical changes, enzyme activity, etc., with little attention given to the irradiation-induced decomposition products. Recently Maxwell and coworkers (7) made a systematic study of the decomposition of a single-solute system, glycine, by 50 kv x-rays. In this work they could identify at least eight decomposition products. Of considerable importance is the biological role of these irradiation-induced decomposition products.

The findings presented here involve a study of the luminescence efficiency of different biological compounds under high-energy gamma bombardment before and after 50 kv x-irradiation. Of interest are the possible effects of the radiation-induced decomposition products on the luminescence efficiency of the biological compounds.

II. EXPERIMENTAL

The experimental arrangement used in these studies is shown in Figure 1. It consists of a lead shield containing a Co^{60} gamma source, a sample changer with 4 ml of the solution or solvent in a porcelain crucible, and a 1P28 photomultiplier tube with a commercial photometer as a recording instrument. Further details of the experimental setup are discussed in a previous report (8).

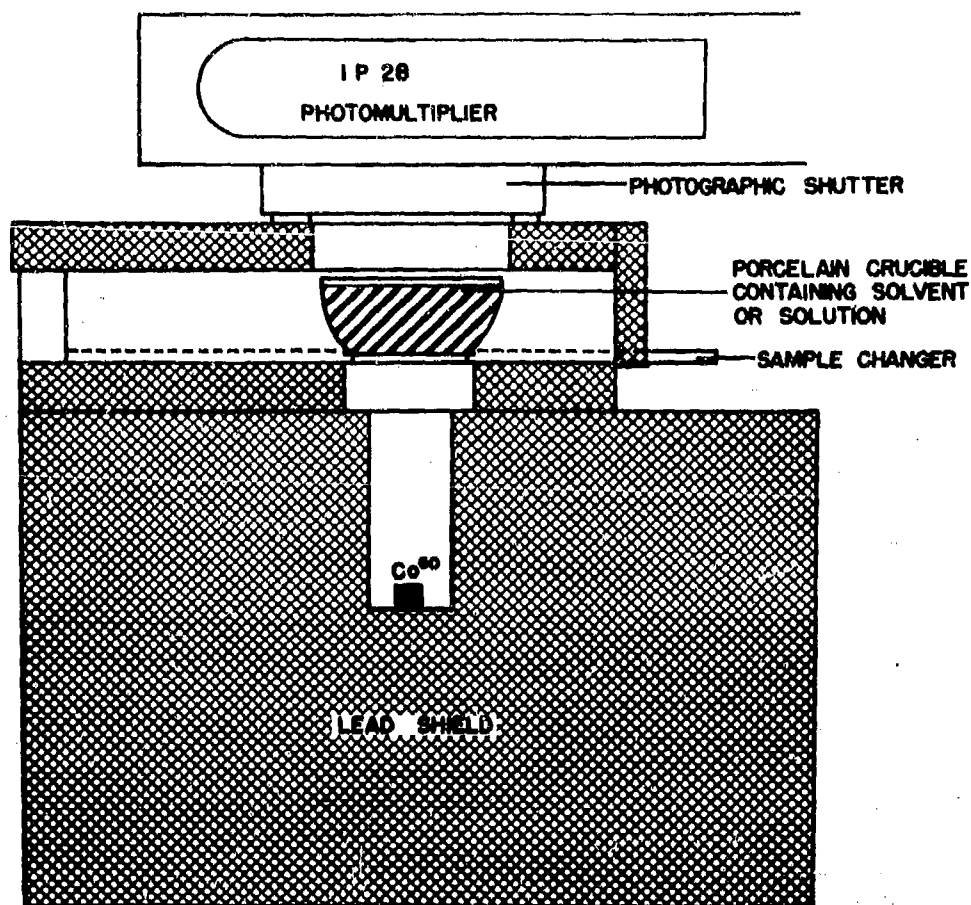


FIGURE 1: EXPERIMENTAL ARRANGEMENT

The biological compounds were generally dissolved in neutral aqueous solutions and the luminescence efficiency was measured relative to the luminescence of the solvent. To study the effect of the concentration of the solute on the luminescence efficiency, different concentrations of the compounds were measured under identical conditions.

The dependence of luminescence efficiency on solution concentration and x-ray dosage was studied. The x-rays were delivered by a thin beryllium-window tube. It was operated at 50 kv and 40 milliamperes without added filtration. An open shallow dish containing 5 ml of a solution of the compound was irradiated at a distance of 5 cm. Under these conditions the surface dose rate was approximately 150,000 r/min in air. The solution was magnetically stirred during the irradiation in order to continually replace the irradiated surface layer and thus increase the yield. Determinations were made of the chemical concentrations of the biological compound in the irradiated solutions for the parallel studies with the luminescence changes.

III. RESULTS

The luminescence efficiency of different biological compounds (amino acids, nucleic acids, vitamins, and enzymes) in aqueous solutions under Co^{60} gamma radiation bombardment in relation to the luminescence of the solvent is presented in Table 1. All substances so far investigated showed lower luminescence efficiency than the solvent, indicating a quenching of the solvent luminescence by the solute molecules.

TABLE 1
LUMINESCENCE OF BIOLOGICAL COMPOUND SOLUTIONS
UNDER Co^{60} GAMMA BOMBARDMENT

SOLUTE	SOLVENT			
	Distilled H_2O		Distilled H_2O + Ammonium Hydroxide	
	pH	%Relative Luminescence	pH	%Relative Luminescence
Distilled H_2O	7.4	100		
Distilled H_2O and Ammonium Hydroxide	10.8	98	10.8	100
		(10^{-2} Molar),		
Glycine	2.0	94	9.8	98
L-Glutamic Acid			9.8	93
L-Methionine	7.2	85	9.8	89
DL-Methionine	7.2	86	9.8	89
L-Phenylalanine	7.0	81	9.8	83
DL-Phenylalanine	7.2	80	9.8	81
Glutathione	3.1	78		
DL-Tryptophane			9.7	79
L-Tryptophane	7.3	68	9.7	76
Glutathione			9.3	72
L-Cystine			9.5	70
Adenylic Acid			9.3	37
Hypoxanthine			9.8	66
Cytosine			10.7	63
Ascorbic Acid	3.3	62	9.8	62
Cytosine	7.5	60		
L-Tyrosine			9.8	59
DL-Tyrosine			9.8	58
Uracil			9.9	58
Thymine			10.0	57
Uric Acid			9.7	56
Crude Catalase (0.8×10^{-5} Molar)	8.8	56		
Crude Catalase (10^{-5} Molar)	7.0	44	10.2	47

Figure 2 shows the dose-effect on the luminescence efficiency for two characteristic compounds, tryptophane and ascorbic acid, exposed to 50 kv x-radiation. In the case of tryptophane x-irradiation decreased the luminescence efficiency, while in the case of ascorbic acid x-irradiation stimulated the light emission. There are also compounds which do not react to x-irradiation. When this condition exists, it may be considered that the compound is not decomposed by the x-rays and/or the loss of the original compound by irradiation may be balanced by the luminescence properties of the formed decomposition products.

To check the function of the experimental arrangement the effect of 50 kv x-rays on anthracene-xylene solutions was studied. There was a decrease in the luminescence efficiency with increasing doses, as shown in Figure 3. This was probably due to the anthracene and/or xylene molecules being damaged by the x-rays. This interpretation finds support in similar studies by Birks (3) using polonium alpha radiation and anthracene crystals. In this case the luminescence efficiency decreased with alpha irradiation and was attributed to the creation of scintillation-inactive centers.

In Figures 4 and 5, the effect of concentration on the x-ray-induced changes in the compounds, catalase, ascorbic acid, and glutathione, is demonstrated. In the crude form of catalase used here there was little difference between the irradiated and the non-irradiated solutions. Ascorbic acid showed an increase in the luminescence after x-irradiation, while irradiated glutathione solutions showed a decrease in luminescence. These apparent changes with concentration denote the complexity of the phenomenon.

Results of parallel studies made on the change in luminescence and on the change in chemical concentration of the solutions after x-irradiation are presented in Figure 6. As shown, values for the change in the molar concentration of the solution and the change in the luminescence efficiency do not coincide for the same dose. Ascorbic acid after five minutes of x-irradiation showed a change of about 60% in luminescence efficiency, whereas the change in the molar concentration was about 92%. The same trend, only on a smaller scale was observed with glutathione. After fifteen minutes of x-irradiation approximately 74% of the original glutathione was destroyed, while the change in luminescence efficiency was only about 50%. These differences may be of interest when considering the role of decomposition products in irradiation experiments.

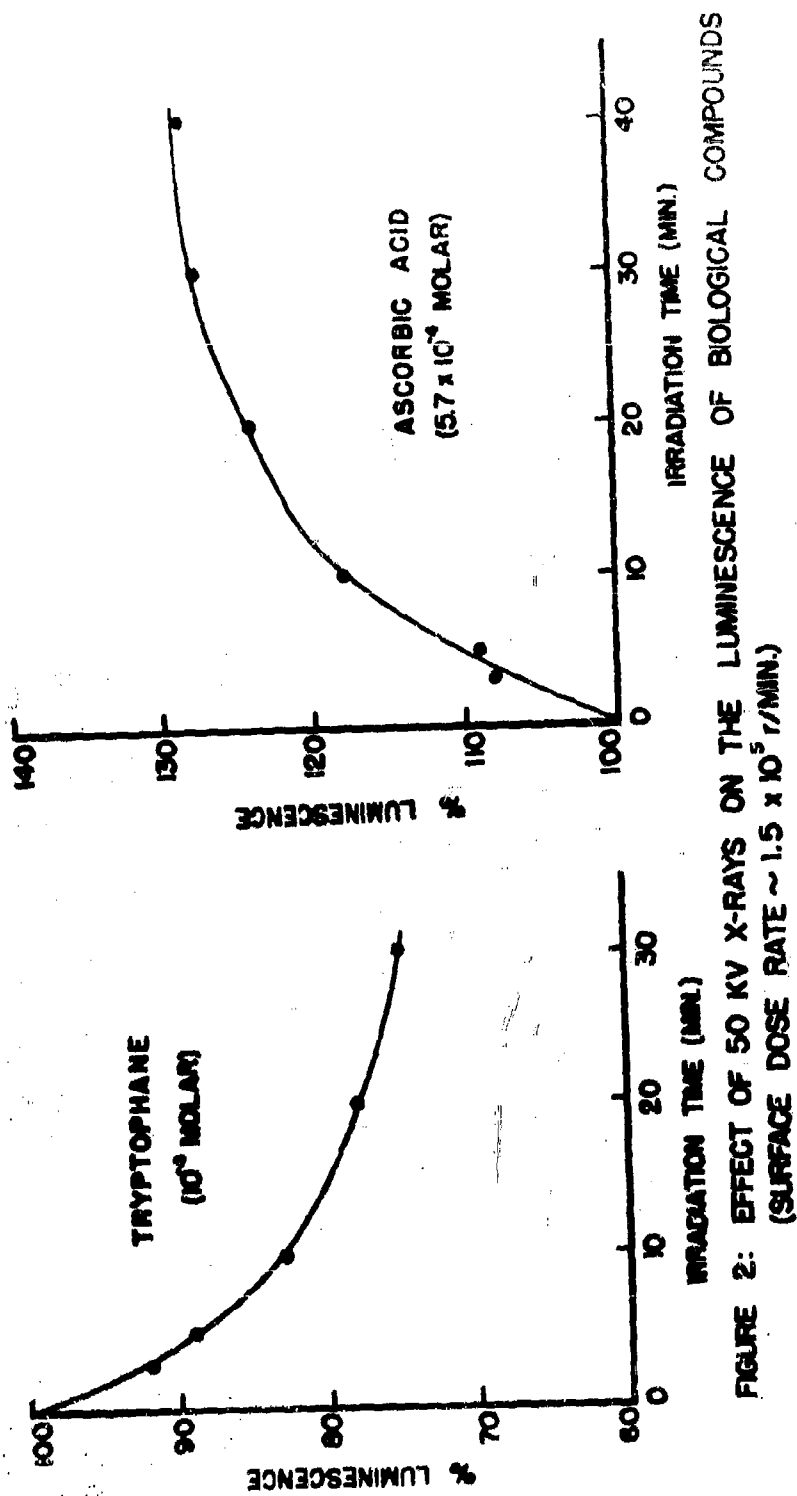


FIGURE 2: EFFECT OF 50 KV X-RAYS ON THE LUMINESCENCE OF BIOLOGICAL COMPOUNDS
(SURFACE DOSE RATE $\sim 1.5 \times 10^5$ r/min.)

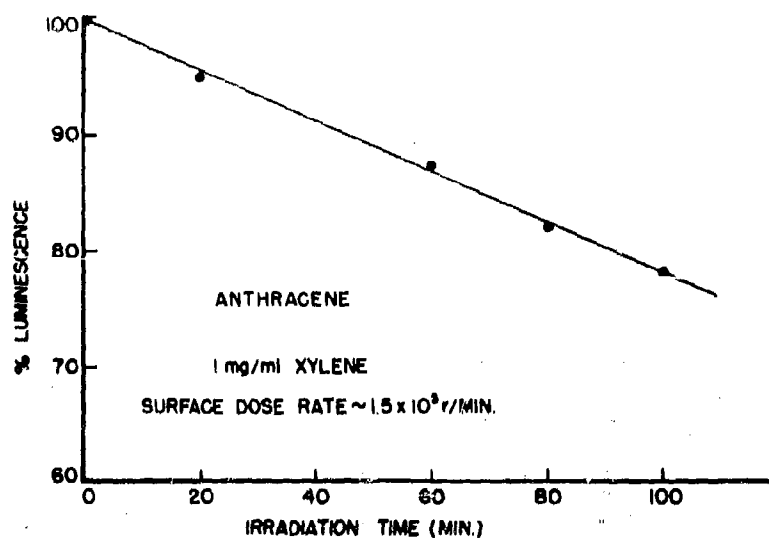


FIGURE 3: EFFECT OF 50 KV X-RAYS ON THE LUMINESCENCE OF ANTHRACENE-XYLENE SOLUTION.

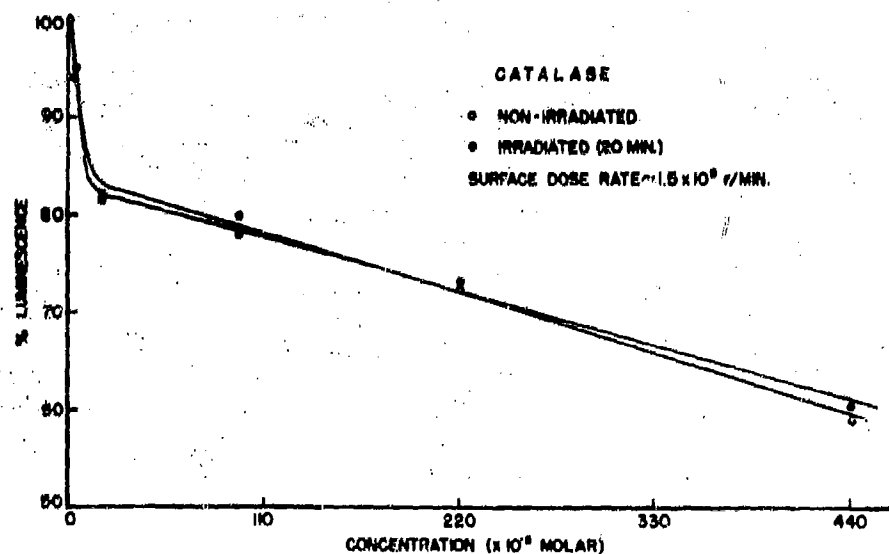


FIGURE 4: EFFECT OF CONCENTRATION ON THE X-RAY INDUCED CHANGES IN THE LUMINESCENCE OF BIOLOGICAL COMPOUNDS.

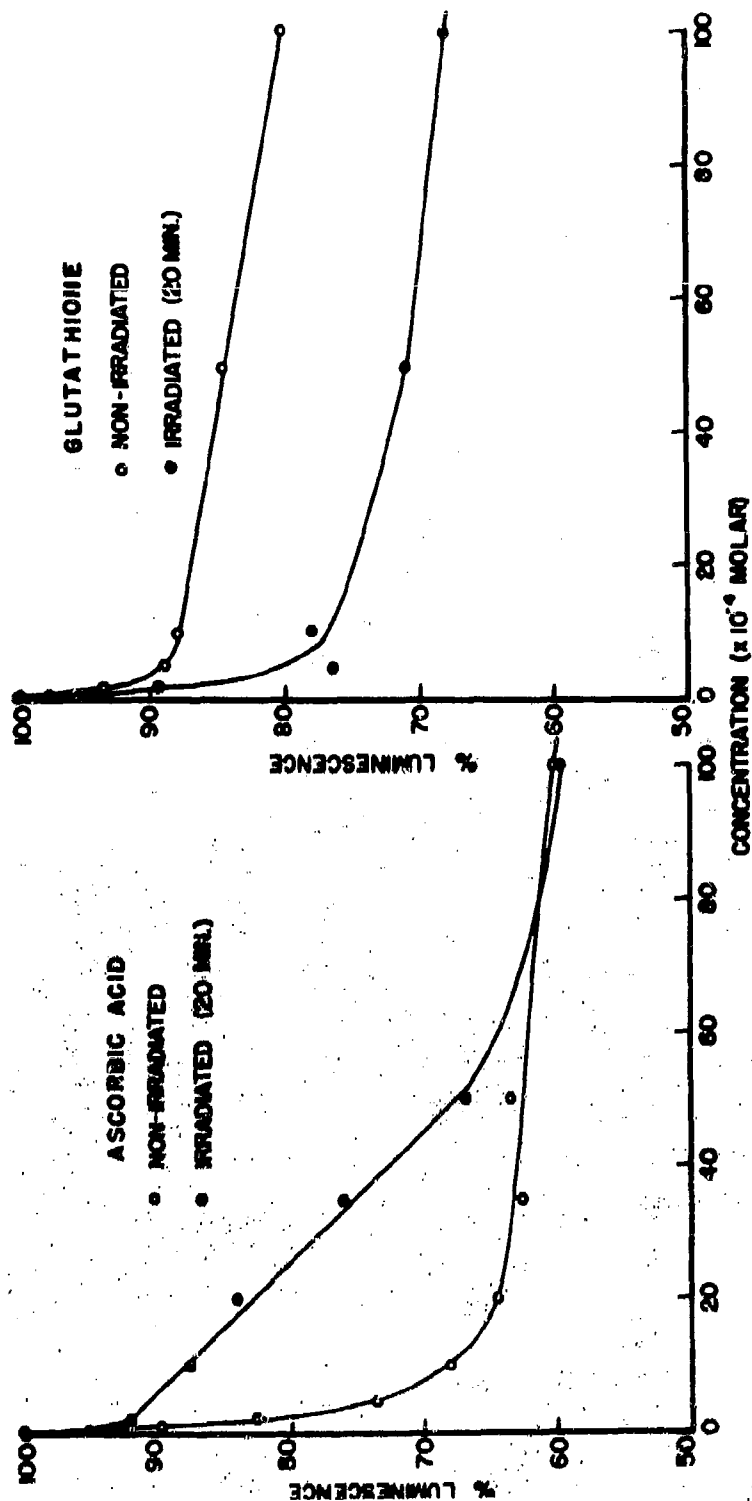


FIGURE 5: EFFECT OF CONCENTRATION ON THE X-RAY INDUCED CHANGES IN THE LUMINESCENCE OF BIOLOGICAL COMPOUNDS (SURFACE DOSE RATE $\sim 1.5 \times 10^5$ r/MIN.)

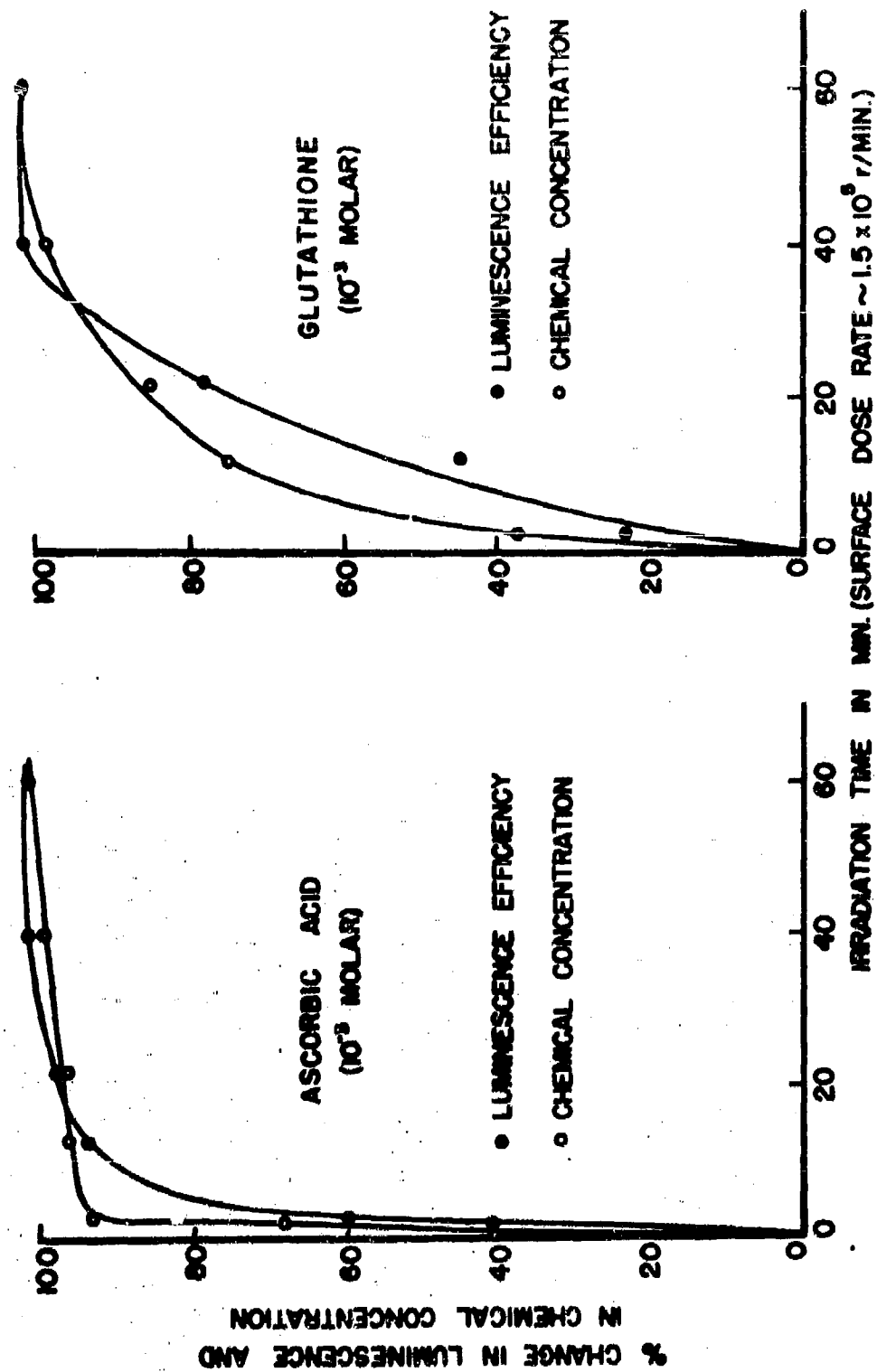


FIGURE 6: PARALLEL STUDY OF CHANGE IN LUMINESCENCE AND IN CHEMICAL CONCENTRATION AFTER 50 KV X-IRRADIATION.

IV. DISCUSSION

With all the biological compounds studied the luminescence efficiency of the solution was lower than that of the solvent. This may be explained by a quenching mechanism, as shown by Kallman and Furst (9), for certain liquid scintillators excited by high-energy bombardment. They classified the various solutions studied into three groups with light outputs relative to a "standard" anthracene crystal. Kallman and Furst's value for distilled water placed it in a group in which all solutes added quenched the luminescence of the solvents. This seems to hold for biological material solutes in a distilled water solvent. These considerations of quenching are further discussed in a previous report (8).

The luminescence efficiency depends on different physical factors (8 and 10) of which concentration seems to be the most important. In all cases studied there was increased quenching with increased concentration.

The compounds observed can be classified into three groups: substances which showed no changes after x-irradiation, substances whose luminescence efficiency increased, and substances whose luminescence efficiency decreased after x-irradiation. Little is known of the biological role of these changes in luminescence efficiency. These findings, however, should be kept in mind when discussing the role of excitations in biological effects.

The irradiation-effect dependence of luminescence on the concentration showed again three principle reactions: no change (catalase), change increasing with concentration and approaching a saturation value (glutathione), and a change going through a maximum with increasing concentration (ascorbic acid). Crude preparations of catalase as reported by Tytell and Kersten (11) did not show inactivation in either dilute or concentrated solutions after eight hours' exposure to soft x-rays, whereas the radiosensitivity of the crystalline preparations increased with the activity of the catalase. Using the crude form of catalase prepared by Armour and Company, 1 mg/cc of catalase was diluted to various molarities and then each dilution irradiated for 20 minutes. The absence of any significant changes in the luminescence efficiency of the x-irradiated sample as compared to the non-irradiated sample may indicate the absence of decomposition products.

The radiation effects on the luminescence efficiency of glutathione and ascorbic acid demonstrate the complexity of the problem. In each case the solutes were decomposed by x-rays as shown by Kinsey (12),

Proctor and Goldblith (13), Barron (14), Alder and Eyring (15), and numerous other investigators. In the case of glutathione where the luminescence efficiency decreased, it may be considered that the decomposition products were able to increase the quenching properties of the solution. With ascorbic acid, which showed an increase in luminescence efficiency, (yet never surpassing that of the solvent), the decomposition products may be considered to have had less efficient quenching properties at certain concentrations.

If the change in the luminescence efficiency curve is to be attributed to the decomposition of the chemical solute by the x-rays, one would expect the curve of the luminescence efficiency to follow closely the decomposition curve. This was not the case.

Another interesting observation was that after an extended period of irradiation both the luminescence efficiency and the chemical concentration showed a 100% change. This may be explained by the total destruction of the primary decomposition products and/or secondary reactions induced in the products arising from the primary irradiation events. The possibility for this action follows from Maxwell's experiments with glycine solutions. In Maxwell's investigations, glycine solutions irradiated with 50 kv x-rays produced primary decomposition products which appreciably underwent secondary reactions. In the course of these reactions the original simple one-solute system changed into a several-solute complex system, for which the recent considerations by Kallman and Furst (16) in energy transfer mechanisms may be applied.

V. CONCLUSIONS

In all biological compounds studied the luminescence efficiency of the solution was lower than that of the solvent under high-energy gamma bombardment. In no case was any biological compound found that increased the luminescence. This quenching of the solvent luminescence by the addition of biological solute molecules is evidence of energy transport from solvent to solute molecules, but utilized in this case perhaps for chemical reactions or converted into heat instead of being re-emitted as light. This may be of prime importance in the biological reaction induced by ionizing radiation.

The absence of any significant changes in the luminescence efficiency of x-irradiated catalase as compared to the non-irradiated catalase indicated the absence of decomposition products. In the case of glutathione, where the luminescence efficiency decreased, it may be considered that the decomposition products were able to increase the

quenching of the solution. With ascorbic acid, which showed an increase in luminescence efficiency (yet never surpassing that of the solvent), the decomposition products may be considered to have had less efficient quenching properties at certain concentrations.

VI. RECOMMENDATIONS

Spectral curves should be obtained for the biological compound solutions under the various x-irradiation conditions studied in an attempt to correlate spectral changes with the luminescence efficiency changes observed.

Parallel studies of percent change in solute chemical concentration and percent changes in luminescence efficiency should be continued to further indicate the role of the irradiation-induced decomposition products.

Other types of radiation should be used to determine their effects on the luminescence efficiency of biological compounds.

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